Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/022165

International filing date: 08 July 2004 (08.07.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/485,503
Filing date: 08 July 2003 (08.07.2003)

Date of receipt at the International Bureau: 13 September 2004 (13.09.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1221527

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

September 05, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/485,503*
FILING DATE: *July 08, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/22165*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

BAKER BOTTS LLP

Please type a plus sign (+) inside this box → ☒

Attorney Docket No. P35892
Express Mail Label No. EV 342493242US

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Bertram	Jacobs	Tempe, Arizona
Chandra	Mitnik	Tempe, Arizona
Langland	Jeffrey	Chandler, Arizona

☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

MUTANTS OF VACCINIA VIRUS AS ONCOLYTIC AGENTS

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☑ Customer Number 21003 → *Type Customer Number here*

OR

Place Customer Number Bar Code Label here

☐ Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS (check all that apply)

☑ Specification *Number of Pages* 10

☑ Drawing(s) *Number of Sheets* 11

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number

☑ Other (specify) 1 PAGE aBSTRACT

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 02-4377

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☑ Yes, the name of the U.S. Government agency and the Government contract number are: Wallace Foundation TDT8786

Respectfully submitted,

SIGNATURE Carmella L. Stephens

TYPED or PRINTED NAME Carmella L. Stephens

TELEPHONE 212 408-2539

Date: July 8, 2003

REGISTRATION NO. (if appropriate) 41,328

Docket Number: P35892

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

BAKER BOTTS L.L.P.

PROVISIONAL APPLICATION COVER SHEET
Additional Page

Docket Number	P35892	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

BAKER BOTTS LLP

FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	
Filing Date	July 8, 2003
First Named Inventor	Jacobs et al.
Examiner Name	
Art Unit	
Attorney Docket No.	P35892

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 02-4377

Deposit Account Name: Baker Botts LLP

The Commissioner is authorized to: *(check all that apply)*

☑ Charge fee(s) indicated below ☐ Credit any overpayments

☑ Charge any additional fee required under 37CFR 1.16 and 1.17

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below		Fee Paid
Total Claims		- 20 =	0	x		= 0
Independent Claims		- 3 =	0	x		= 0
Multiple Dependent						=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY

(Complete *(if applicable)*)

Name (Print/Type)	Carmella L. Stephens	*Registration No. (Attorney/Agent)*	41,328	*Telephone*	212 408-2539
Signature	Carmella L. Stephens			*Date*	July 8, 2003

CERTIFICATION UNDER 37 C.F.R. 1.8(a) OR 1.10*

(When using Express Mail, the Express Mail label number is mandatory; Express Mail Certification is optional.)

I hereby certify that, on the date shown below, this correspondence is being:

MAILING

☑ deposited with the United States Postal Service in an envelope addressed to the Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.

37 C.F.R. 1.8(a)

☐ with sufficient postage as first class mail.

37 C.F.R. 1.10*

☑ as "Express Mail Post Office to Address"

Mailing Label No. EV 342498242US (mandatory)

Leroy Chick

Signature

Date: July 8, 2003

Leroy Chick

(type or print name of person certifying)

***WARNING:** *Each paper of fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing 37 C.F.R. 1.10(b). "Since the filing of correspondence under § 1.10 without the Express Mail label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition. "Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

FILE NO. P35892

BAKERBOTTS L.L.P.

30 ROCKEFELLER PLAZA

NEW YORK, NEW YORK 10112

TO ALL WHOM IT MAY CONCERN:

Be it known that WE, Bertram Jacobs, Chandra Mitnik, and Jeffrey Langland, a citizens of the United States, whose post office addresses are, 1004 S. Wilson Street, Tempe, Arizona 85281, 1056 E. Sandpiper Drive, Tempe, Arizona 85283, and 506 W. El Alba Way, Chandler, Arizona 85225, respectively, have invented an improvement in:

MUTANTS OF VACCINIA VIRUS AS ONCOLYTIC AGENTS

of which the following is a

SPECIFICATION

INTRODUCTION

The present invention relates to mutant oncolytic vaccinia viruses and their use for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region. The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are capable of replication in oncogenic *ras* expressing cells resulting in cell lysis. The invention further provides methods for treating *ras*-mediated proliferative disorders, such as neoplasms, in a host comprising administration of mutant vaccinia virus under conditions which result in substantial lysis of the proliferating cancer cells.

BACKGROUND OF INVENTION

Most current cancer treatments have some selectivity for cells that divide rapidly, such as cancer cells, intestinal cells, and hair follicle cells, but ultimately fail to take advantage of the molecular differences between tumor and normal cells. Oncolytic ("onco" meaning cancer, "lytic" meaning killing) viruses represent a promising new cancer therapy that seeks to exploit the natural properties of viruses to aid in the fight against cancer. Oncolytic viruses are viruses that infect and replicate in cancer cells, destroying the cancer cells and leaving normal cells largely unaffected. Such viruses include reoviruses (Wilcox et al., 2001, J. Natl. Cancer Inst. 93:903-912; Coffey et al., 1998, Science 2:83:1332-1331; Norman et al., 2002, Human Gene Therapy 13:641-642; Strong et al., 1998, 12:3351-3362), vesicular stomatitis virus (VSV) (Stojdl, 2000 nature 6:821-825), herpes simplex virus (HSV) (Farasetti et al., Nature Cell Biology 3:745) and human influenza A virus (Bergmann et al., 2001 Cncer Research 64:8188-8193).

The *ras* protein plays a central role in a variety of cellular processes in vertebrates and invertebrates. Active *ras*, through a kinase cascade, is responsible for cell differentiation and proliferation in response to normal mitogenic signals. A mutation in the *ras* gene can cause uncontrolled cell growth, leading to tumor formation. It has been demonstrated that a large number of tumors contain a mutated *ras* gene that results in a constitutively expressed or always active form of *ras*, thus proving to be an effective genetic marker of tumor cells and a potential attractive target for therapy.

In addition to these cell growth activities, the *ras* pathway alters the anti-viral interferon pathway. The interferon system acts as an alarm for the host by warning nearby cells

of an impending virus attack. After a cell receives the warning signal of interferon, a biochemical cascade is activated resulting in the induction of hundreds of genes. Among these genes induced by interferon, is the well-studied antiviral dsRNA-dependent protein kinase (PKR). This enzyme becomes activated in the presence of the double-stranded RNA produced during most viral infections. The activated PKR inhibits protein synthesis in order to halt the viral infection. The *ras* pathway results in an increase in an inhibitor of PKR, which effectively blocks this step in the interferon pathway. This inhibitor has been termed RIKI, which stands for *ras*-inducible PKR kinase inhibitor. RIKI is believed to be associated with a weak tyrosine or serine/threonine phosphatase activity. Thus, it disables PKR by dephosphorylation, leading to an inactive form of PKR.

Many viruses, including vaccinia virus, have developed mechanisms in order to evade the host defense system, specifically the actions of interferon. One of the ways that vaccinia virus subverts the host immune response is by encoding the protein E3L. As noted above, PKR becomes activated by the double-stranded RNA produced during viral infections. Vaccinia virus masks the double-stranded RNA it produces with the E3L protein, which binds and sequesters double-stranded RNA. The masked double-stranded RNA cannot activate PKR. Consequently, viral protein synthesis continues even in the presence of interferon, and the viral infection proceeds unimpeded.

Use of vaccinia virus as an oncolytic agent offers several advantages over other oncolytic viruses. First, the viruses can be genetically engineered with ease. Thus, by inserting or deleting genes from vaccinia, the safety and efficacy of the virus can be enhanced. An additional advantage is the wide base of knowledge concerning vaccinia virus infections in

humans. Finally, vaccinia virus has been shown to be safe in all but immunocompromised individuals.

By creating various deletion mutants in the vaccinia virus E3L region, viruses have been created that are lacking in their ability to inhibit PKR and, thus, become dependent on the PKR inhibitory activity found in *ras*-transformed cells. Such viruses provide a means for targeting selective cell lysis to *ras* transformed cells.

SUMMARY OF THE INVENTION

The present invention relates to mutant oncolytic vaccinia viruses and the use of such viruses for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region.

The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are able to replicate in oncogenic *ras* expressing cells resulting in cell lysis. As demonstrated herein, several mutant vaccinia viruses are shown to be oncolytic with specificity for a particular molecular pathway that is commonly dysregulated in a variety of cancers. These vaccinia viruses are dependent on the overexpression of *ras*, a key molecular characteristic of over 50% of cancers. Thus, the present invention provides methods for treating *ras*-mediated proliferative disorders in a host wherein said method comprises administration of mutant vaccinia virus under conditions which result in substantial lysis of proliferating cancer cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Deletion mutants of E3L in vaccinia virus and their PKR inhibitory and *ras* dependency characteristics.

Figure 2. NIH-3T3 or NIH-3T3 overexpressing the ras protein were either mock infected or infected with the above identified vaccina virus constructs at an MOI (multiplicity of infection) of 0.01. Cytopathic effect is a description of any adverse properties of cells following infection. Photographs were taken at 24, 48 and 72 hours post infection to record cytopathic effect. In Figure 2A, all cells were mock infected and appear normal and healthy through 72 hours post infection. In Figure 2B, cells were infected with wt WR virus, which is not ras dependent. Cytopathic effect was noted in both the NIH-3T3 and NIH-3T3 Ha-Ras beginning at 48 hours post infection and continuing to 72 hours post infection. In Figure 2B, cells were infected with wt WR virus, which was not ras –dependent. Cytopathic effect was noted in both NIH-3T3 ans NIH-3T3 Ha-Ras beginning at 48hours post infection and continuing to 72 hours post infection. Slight cytopathic effect was noted in Figure 2E, when cells were infected with WRdel7C, indicating that this virus is less ras-dependent than the other mutant viruses. Cytopathic effect was not evident in Figures 2C, 2D and 2F in the NIH-3T3 cells, indicating that these virus constructs are *ras*-dependent.

Figure 3. This figure represents viral replication over a 72-hour period. NIH-3T3 or NIH-3T3 Ha-Ras cells were infected with wtWR, WRde183N, WRdel54N, and WRdelE3L at an MOI of 0.01. Viral replication was measured by determining how many infectious virus particles were present after 72 hours. The number of infectious virus particles is expressed as

titer and is on the y-axis, while the various vaccinia constructs are depicted on the x-axis. WtWR grew to high titers in both cell lines. Titers dropped in the NIH-3T3 cells, but remained high in the NIH-3T3 Ha-Ras cells for all of the vaccinia constructs.

Figure 4. This figure represents viral replication over a 72-hour period. Either normal breast cells or cancerous breast cells were infected with wtWR, WRde154N, and WRdelE3L at an MOI of 0.01. Viral replication was measured by determining how many infectious virus particles were present after 72 hours. The number of infectious virus particles is expressed as titer and is on the y-axis, while the various vaccinia constructs are depicted on the x-axis. WtWR grew to high titers in all cell lines. WRdelE3L failed to grow in any cell line. WRde154N did not grow in the normal breast cells, or in two of the cancer cell lines. However, WRde154N grew to high titers in four out of six breast cancer cell lines.

Figure 5. This figure depicts viral replication by measuring protein synthesis. NIH-3T3 or NIH-3T3 Ha-Ras cells were either mock infected or infected with wtWR, WRde183N, WRde154N, WRde126C, or WrdelE3L. At 72 hours post infection, the cells were harvested and their proteins loaded onto this gel. This gel was then probed with antibodies against vaccinia virus in order to detect vaccinia virus proteins. Vaccinia virus proteins were not detected in either mock infection. Vaccinia virus proteins were detected in wtWR and less in WRde183N infected NIH-3T3 cells. Viral protein synthesis was not detected in WRde154N, WRde126C, or WrdelE3L infected NIH-3T3 cells. Viral protein synthesis was detected in all infected NIH-3T3 Ha-Ras cells, with lower levels noted in WRde154N infected cells.

Figure 6. To confirm the endogenous inhibition of PKR in *ras*-transformed cells, a PKR phosphorylation assay was conducted. NIH-3T3 or NIH-3T3 Ha-Ras cells were either incubated with interferon to induce production of PKR or were not incubated. The cells were harvested and subjected to an *in vitro* kinase assay. Briefly, cell lysates were incubated with or without double-stranded RNA to activate the PKR and radioactively labeled substrate to detect the phosphorylation event (representing PKR activation). These lysates were purified and loaded onto a gel, that was subsequently exposed to x-ray film to detect any radioactive PKR. The intensity of each PKR band was measured using the computer software ImageQuant, and the relative intensities were graphed. High levels of activated PKR were detected in NIH-3T3 cells that were incubated with both double-stranded RNA and interferon. The effect was dampened in the NIH-3T3 Ha-Ras cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to mutant oncolytic vaccinia viruses and the use of such viruses for selective destruction of cancer cells. The mutant vaccinia viruses of the invention comprise mutant vaccinia viruses with a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). In particular, the present invention provides recombinant vaccinia virus from which the region encoding the E3L gene product has been inactivated. Such inactivation may result from partial or complete deletion of the E3L region or, alternatively, substitution of nucleotides within the E3L region that result in inactivation of the E3L gene product. The invention is based on the discovery that such mutant viruses are unable to inhibit PKR thus rendering the viruses dependent on the PKR inhibitory activity found in *ras*-transformed cells.

The E3L gene product of the vaccinia virus is a 190 amino acid polypeptide. The E3L gene codes for several functions including a dsRNA-binding protein, a Z-DNA-binding protein, and dimerization. Amino acids 118-190 have been implicated in dsRNA binding, as disclosed by Chang and Jacobs (1993, *Virology* **194**:537-547). Amino acid numbering as used herein is adopted from Goebel et al., 1990, *Virology* **179**:247-66, 577-63.

According to the invention "deletion of the E3L gene" and its grammatical equivalents refer to a vaccinia virus wherein a nucleic acid encoding all 190 amino acids or a subset of the 190 amino acids of E3L are not present. According to the invention, if the vaccinia virus having a deletion in the E3L gene has a residual nucleic acid encoding a subset of the 190 amino acids of E3L, said residual nucleic acid is incapable of producing a functional gene product or the gene product is incapable of binding dsRNA. The ability of the E3L gene product to bind to dsRNA can be determined by binding assays known in the art and disclosed, for example, by Chang et al., 1993, Virology 194:537.

Deletion of the E3L gene from vaccinia virus results in a virus that is interferon-sensitive, but also is highly debilitated for replication in many cells in culture (Jacobs and Langland, 1996, *Virolology* **219**(2):339-349). However, as demonstrated herein, such viruses are capable of replication in *ras*-transformed cells thereby providing a method for targeted cell lysis of *ras*-transformed cells.

The recombinant vaccinia virus of the present invention may be constructed by methods known in the art, and preferably by homologous recombination. Standard homologous recombination techniques utilize transfection with DNA fragments or plasmids containing

sequences homologous to viral DNA, and infection with wild-type or recombinant vaccinia virus, to achieve recombination in infected cells. Conventional marker rescue techniques may be used to identify recombinant vaccinia virus. Representative methods for production of recombinant vaccinia virus by homologous recombination are disclosed by Piccini et al., 1987, Methods in Enzymology 153:545.

[0001] For example, the recombinant vaccinia virus of a preferred embodiment of the present invention may be constructed by infecting host cells with vaccinia virus from which the E3L gene has been deleted. The vaccinia virus used for preparing the recombinant vaccinia virus of the invention may be a naturally occurring or engineered strain. Strains useful as human and veterinary vaccines are particularly preferred and are well-known and commercially available. Such strains include Wyeth, Lister, WR, and engineered deletion mutants of Copenhagen such as those disclosed in U.S. Patent 5,762,938. Recombination plasmids may be made by standard methods known in the art. The nucleic acid sequences of the vaccinia virus E3L gene and the left and right flanking arms are well-known in the art, and may be found for example, in Earl et al., 1993, in *Genetic Maps: locus maps of complex genomes*, O'Brien, ed., Cold Spring Harbor Laboratory Press, 1.157 and Goebel et al., 1990, *supra*. The amino acid numbering used herein is adopted from Goebel et al., 1990, *supra*. The vaccinia virus used for recombination may further comprise other deletions, inactivations, or exogenous DNA.

[0002] The present invention further provides compositions for use in targeted cell lysis wherein said compositions comprise a recombinant vaccinia virus, or viral vector, and a carrier. The term carrier as used herein includes any and all solvents, diluents, dispersion media,

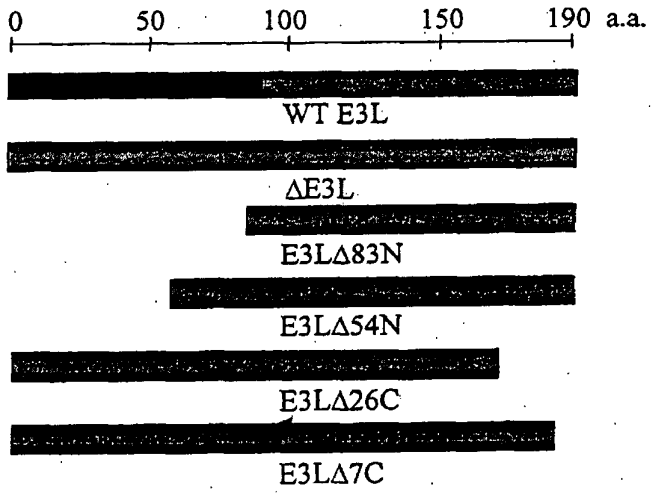
antibacterial and antifungal agents, microcapsules, liposomes, cationic lipid carriers, isotonic and absorption delaying agents, and the like. Suitable carriers are known to those of skill in the art. The compositions of the invention can be prepared in liquid forms, lyophilized forms or aerosolized forms. Other optional components, e.g., stabilizers, buffers, preservatives, flavorings, excipients and the like, can be added.

Also included in the invention is a method of treating a host with cancer, including but not limited to mammals such as a humans, with the novel compositions of the invention under conditions which result in substantial lysis of the proliferating cancer cells. In the method of the invention, the recombinant vaccinia viruses of the invention are administered to *ras*-mediated transformed cells in the host. The compositions, including one or more of the recombinant vaccinia viruses described herein, are administered using routes typically used for such administration, *i.e.*, intravenously, intravascularly, injection at site of tumor, in a suitable dose. The dosage regimen involved in the method of treating, including the timing, number and amounts of treatments, will be determined considering various hosts factors, *e.g.*, the age of the patients, time of administration and type and severity of the cancer.

Various publications are cited herein, the contents of which are hereby incorporated by reference in their entireties herein.

ABSTRACT

The present invention relates to mutant oncolytic vaccinia viruses and their use for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region. The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are capable of replication in oncogenic *ras* expressing cells resulting in cell lysis. The invention further provides methods for treating *ras*-mediated proliferative disorders, such as neoplasms, in a host comprising administration of mutant vaccinia virus under conditions which result in substantial lysis of the proliferating cancer cells.



	PKR inhibition	*ras* dependency
WT E3L	+++	-
ΔE3L	-	+++
E3LΔ83N	++	+
E3LΔ54N	+	++
E3LΔ26C	+	++
E3LΔ7C	+	++

Figure 1. Deletion mutants of E3L in vaccinia virus and their PKR inhibitory and *ras* dependency characteristics.

Figure 2a: Mock infected

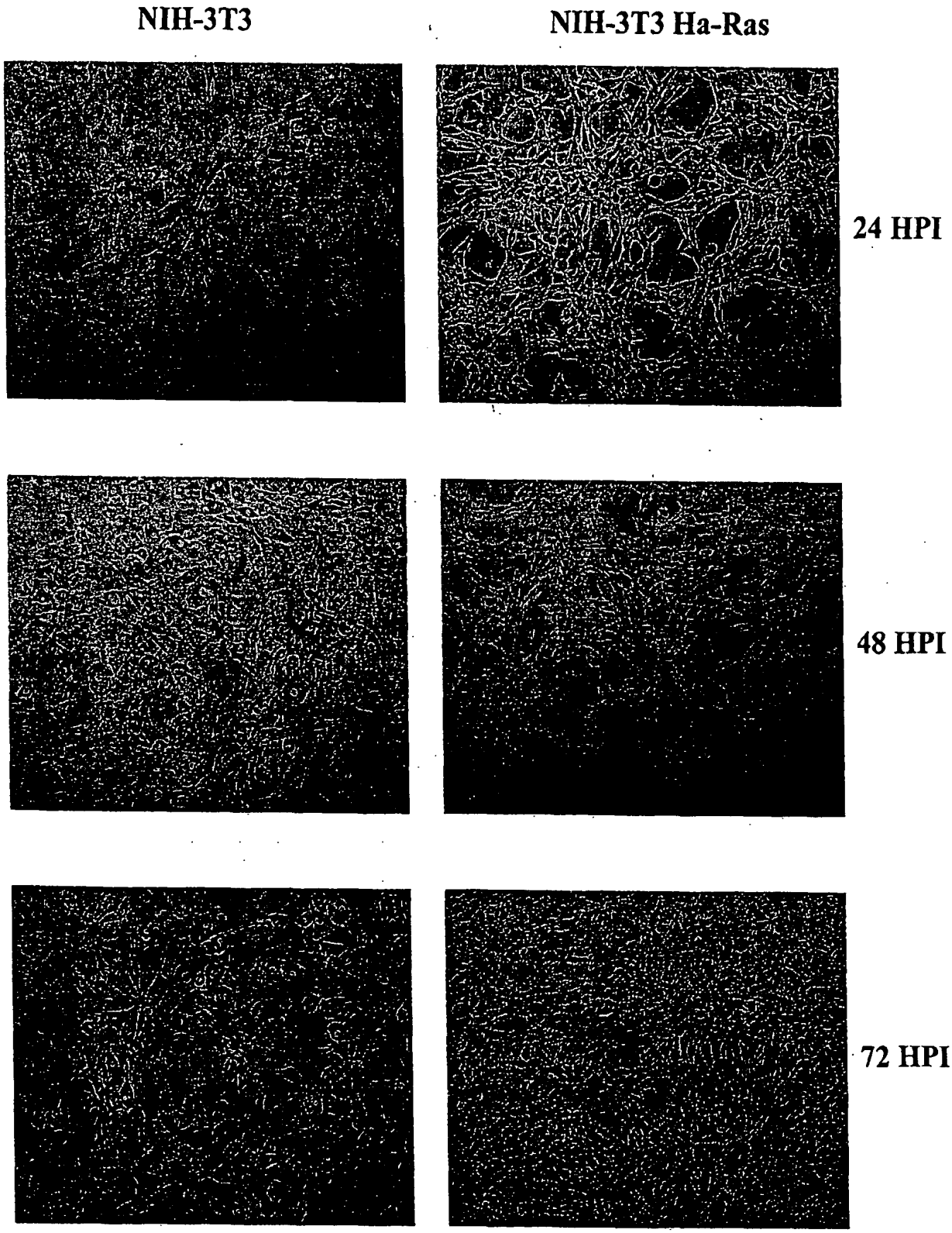


Figure 2b: wtWR

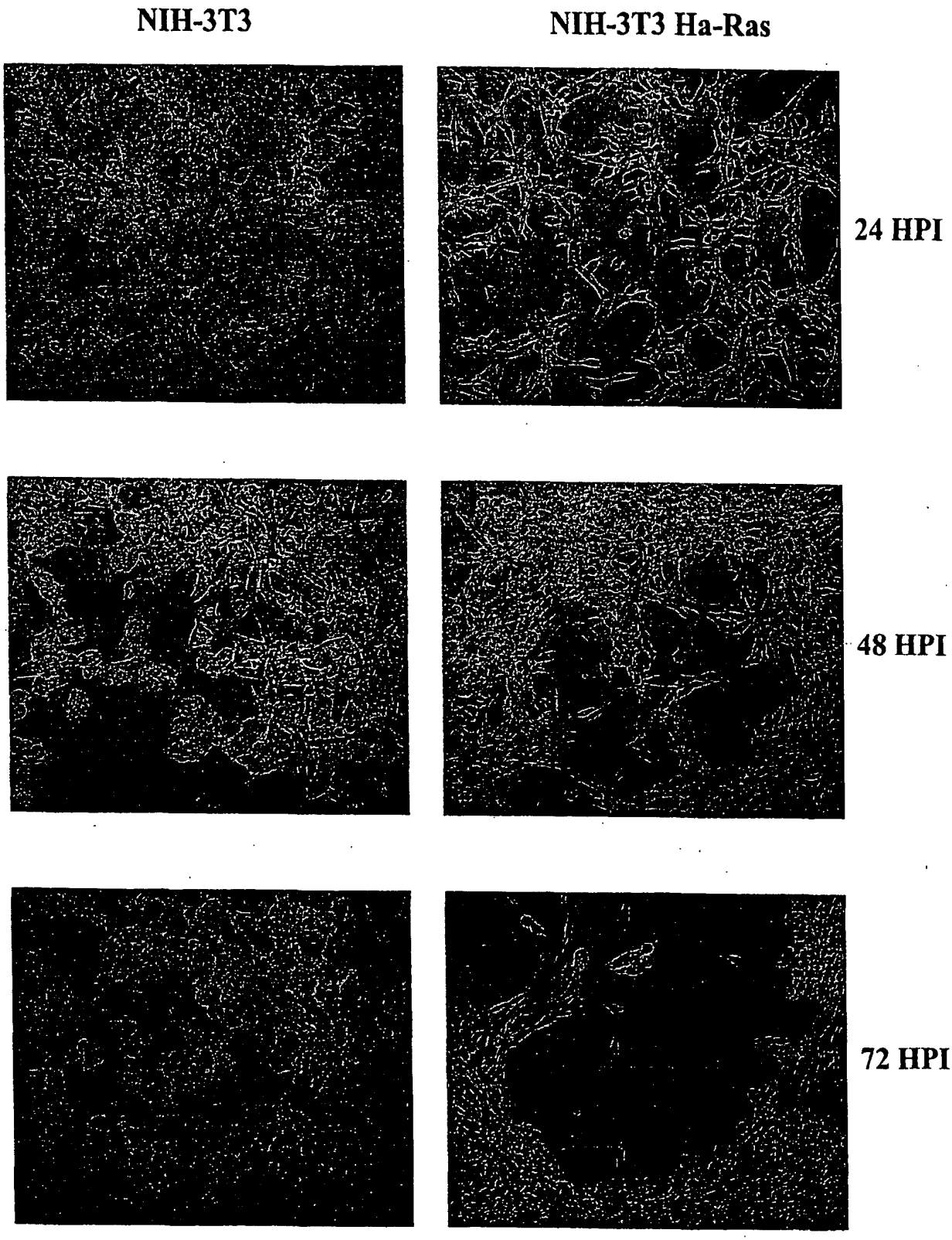


Figure 2c: WRdel83N

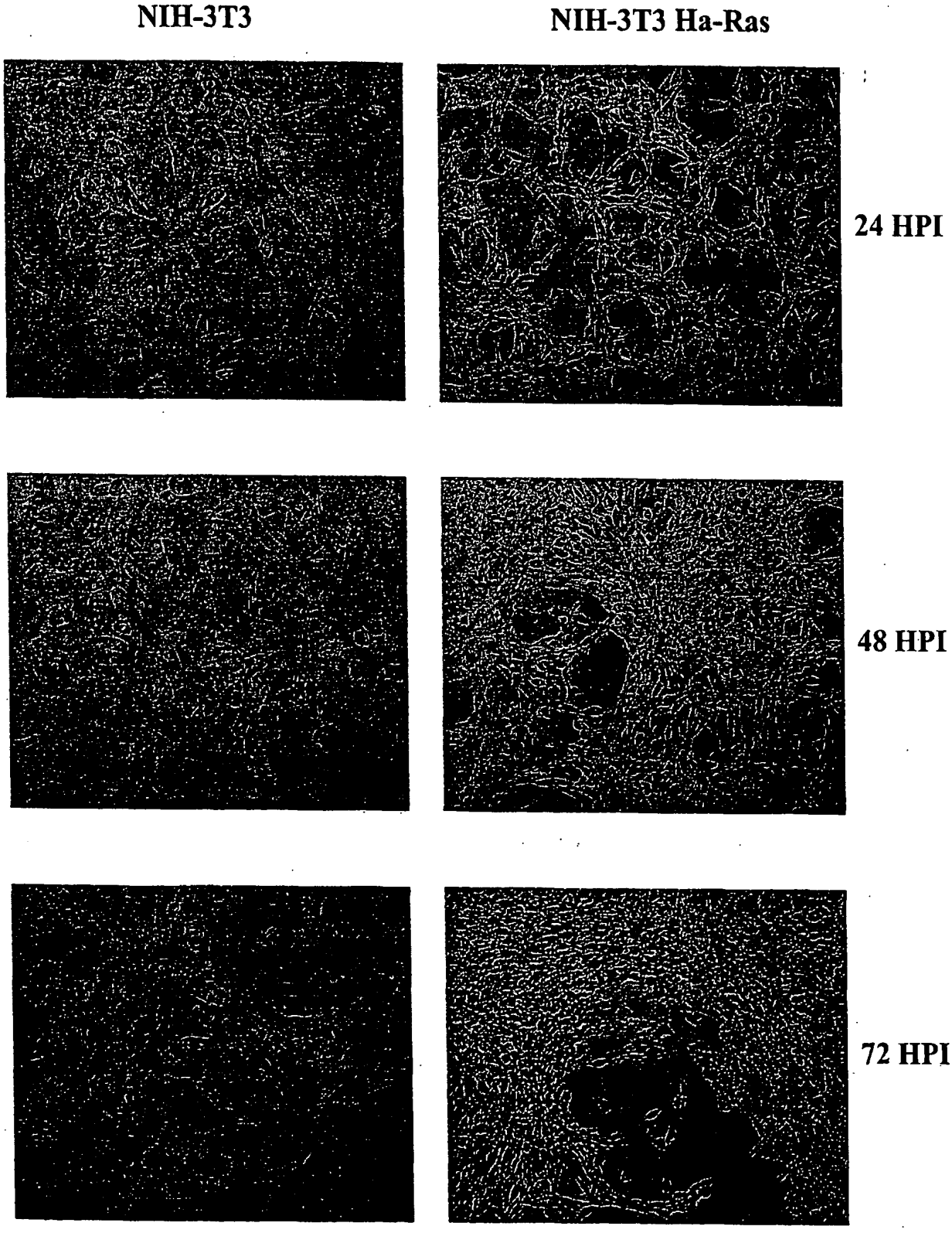


Figure 2d: WRdel54N

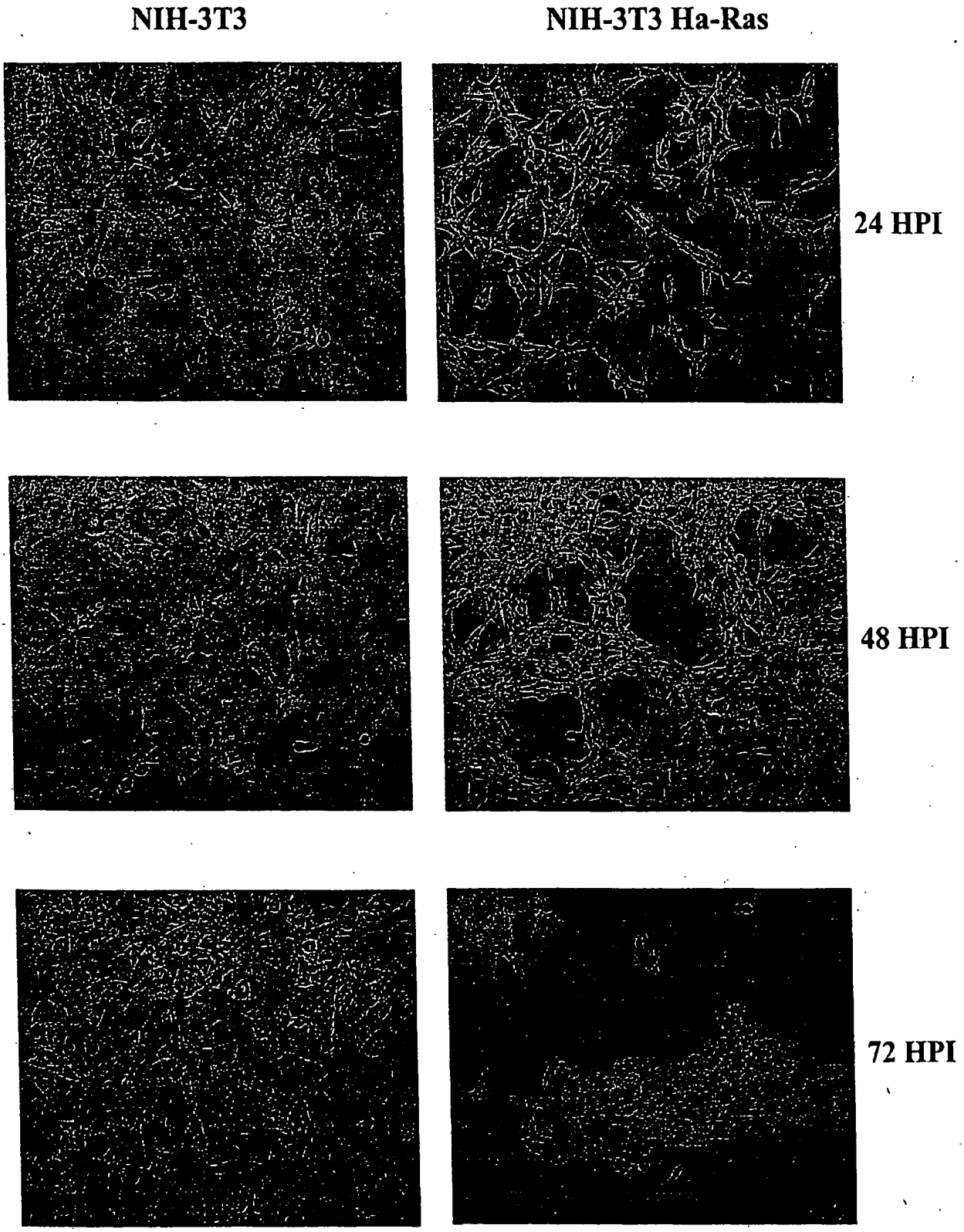


Figure 2e: WRdel7C

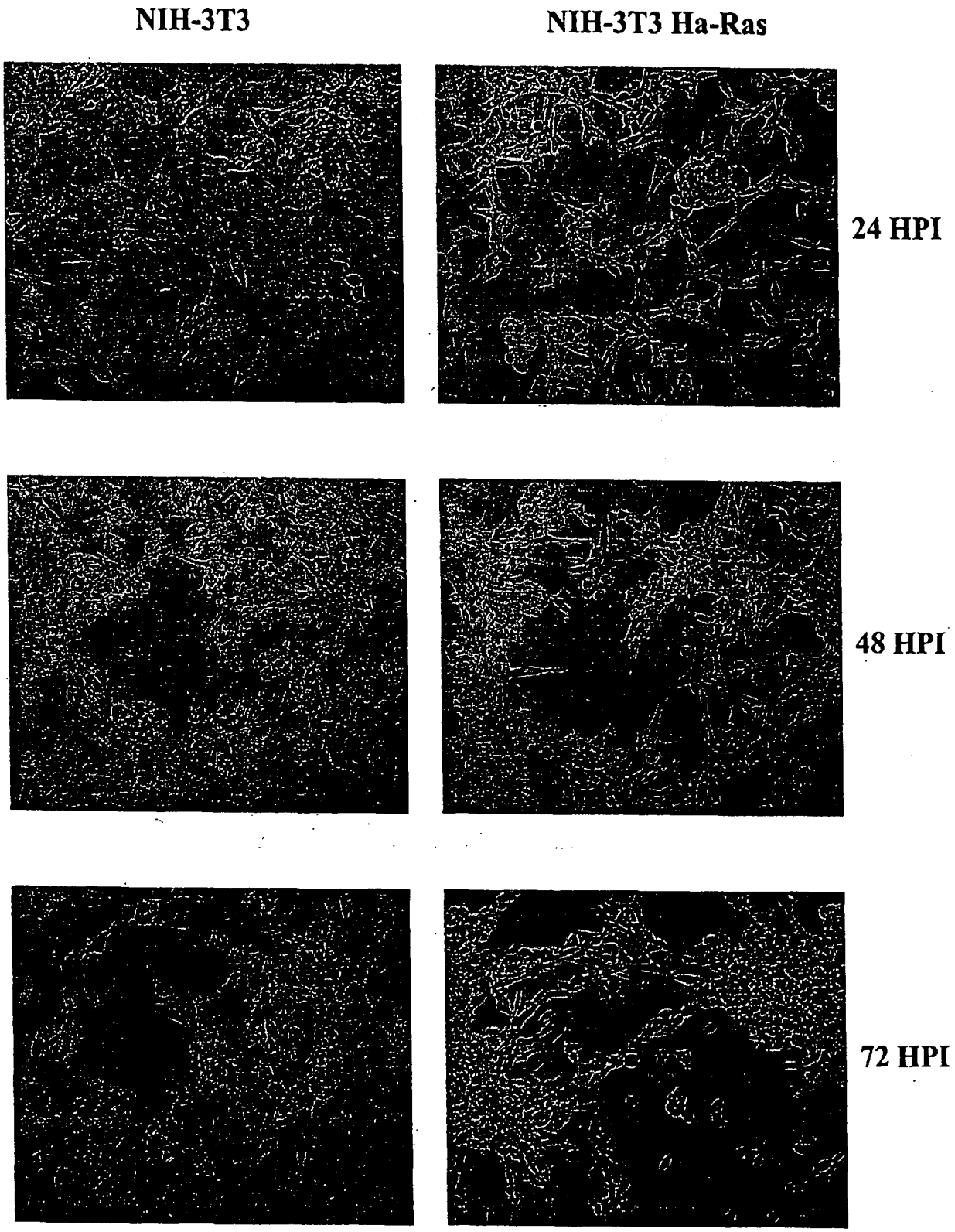


Figure 2f: WRdelE3L

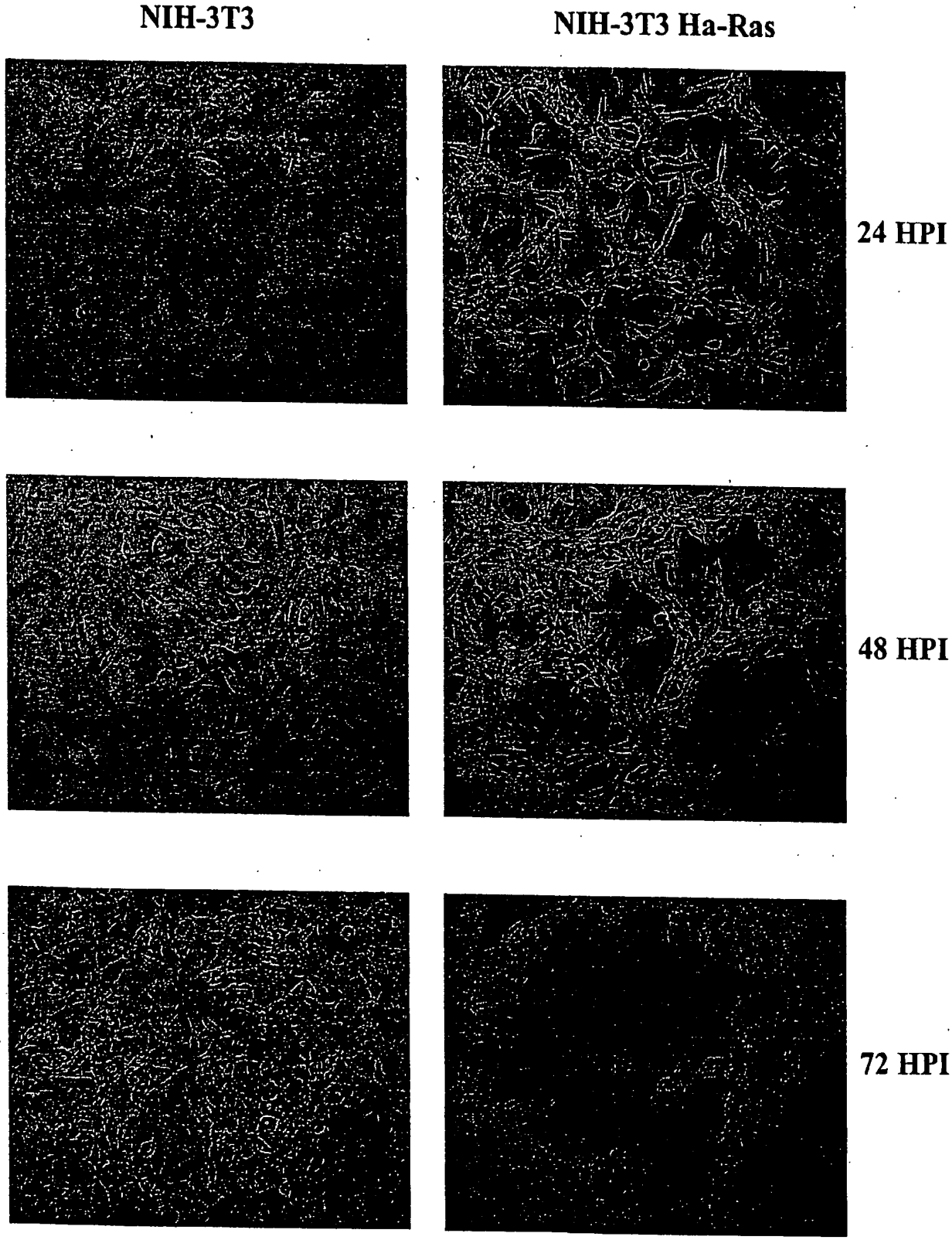


Figure 3: Multi-Step Growth Curve

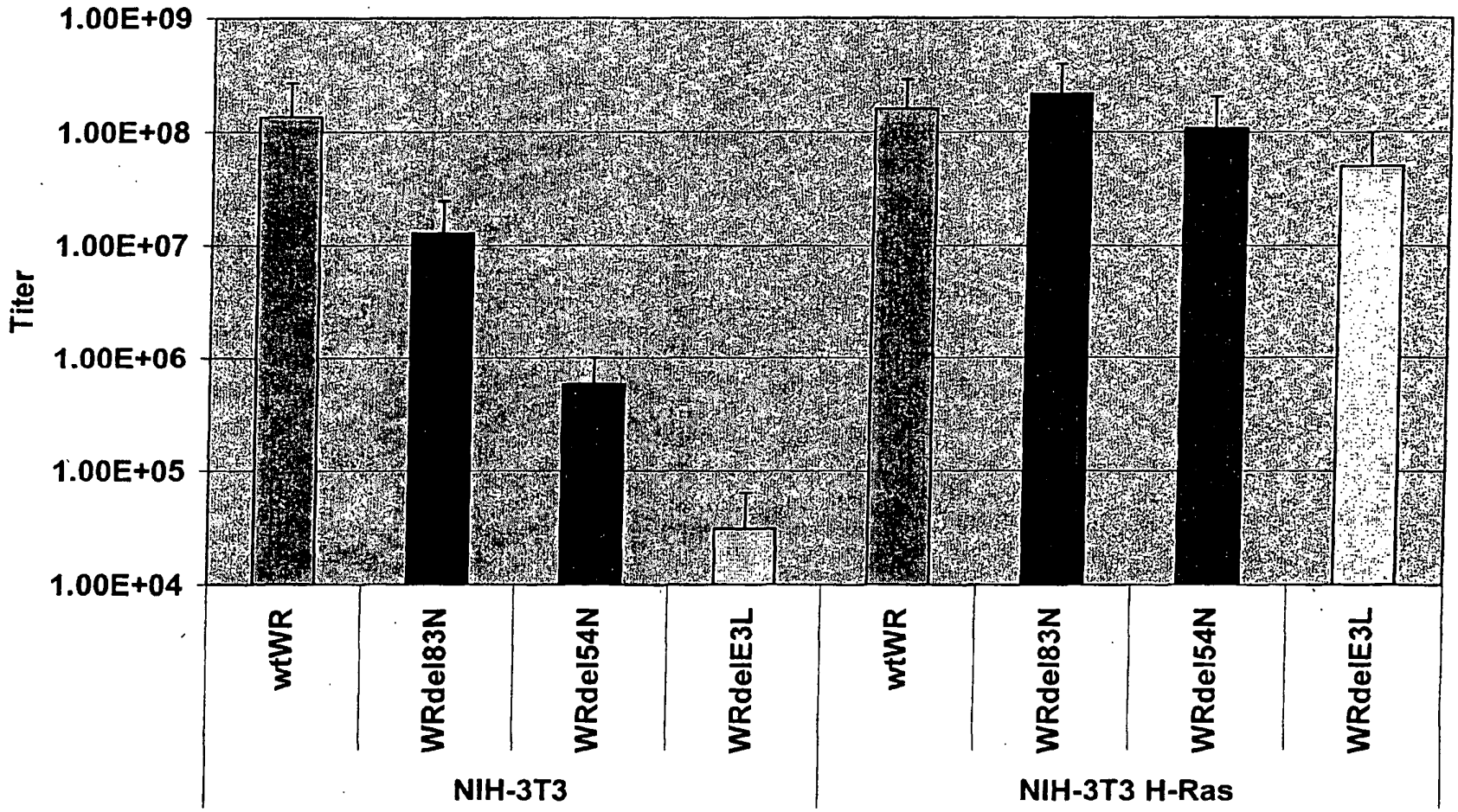
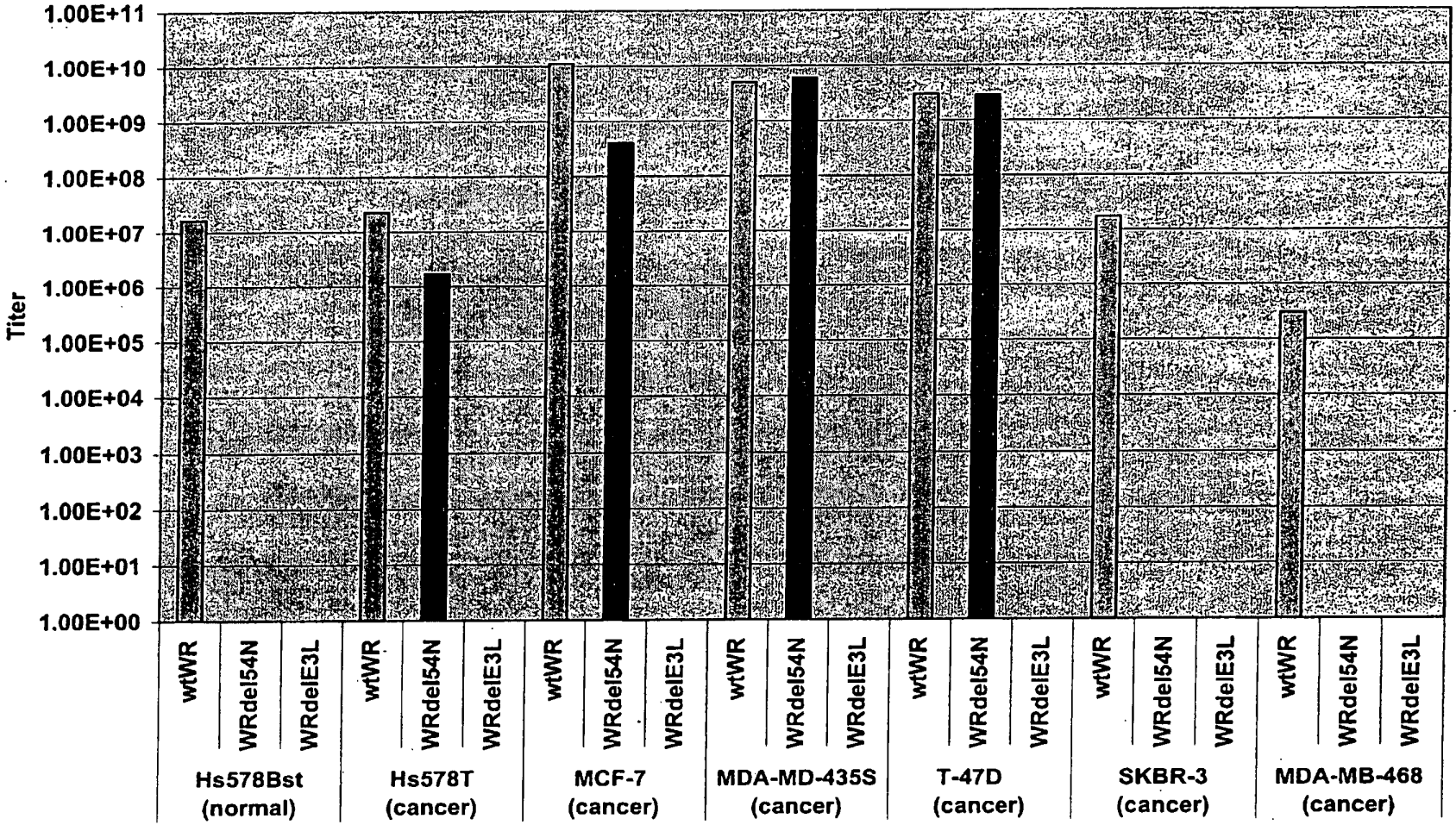


Figure 4: Multi-Step Growth Curve



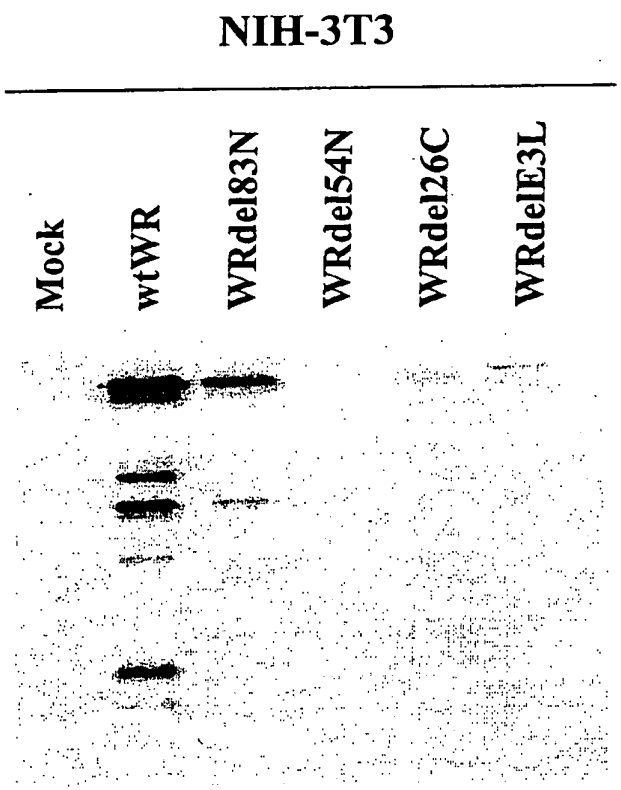


Figure 5a

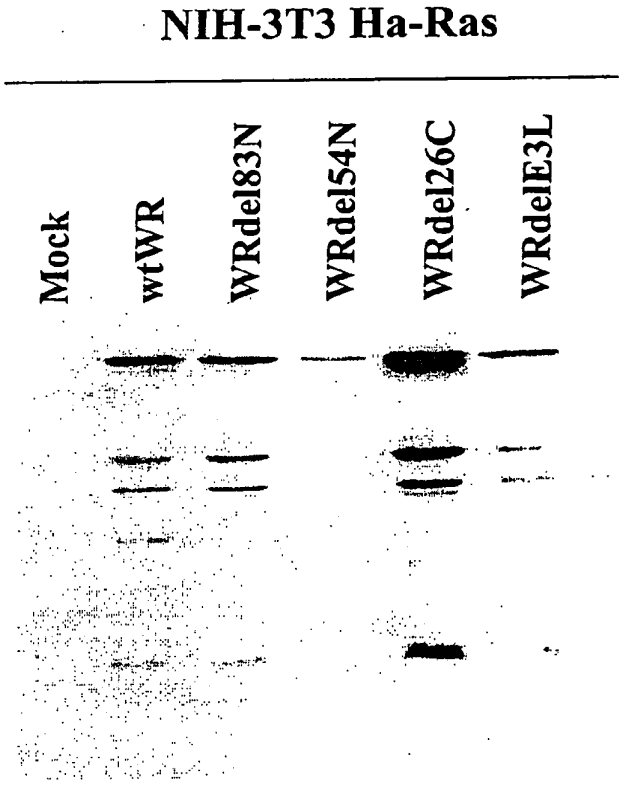


Figure 5b

Figure 6: PKR-P Assay